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<p>This project will be initiated by the establishment of a culture collection isolated from contaminated drag strip soil (DSS) and clean Hudson River Sediment (HRS). Careful isolation, characterization, and long term maintenance of these bacteria and protists is critical for the success of the project. Bacteria will be characterized by sole carbon source utilization as well as standard morphological and chemical characteristics. Clonal cultures of protists will be identified by staining of morphological features for light microscopy, and characterized for their feeding and growth on the bacterial isolates obtained. Stable consortia of bacteria and protists in biphenyl cultures will be established and characterized. Retrieval of frozen consortia of bacteria and protists will be assessed. In addition, protists will be characterized for their sensitivity to biphenyl and Aroclors(R), and assayed for acquired resistance. Studies of sorption and transfer for Aroclors(R), in bacteria and protist cells will be conducted. This very basic microbial ecology work is time consuming, but is essential to lay the ground work for future experiments. Analysis of the role of protists in situ biodegradation will begin with inhibition and/or stimulation of native bacteria and protist populations. Experiments to determent</p>					
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the fate of *Alcaligenes eutrophus* H850 in soil samples with and without protists will also begin. The effects of nutrient limited growth and predation pressure as pre-adaptations to inoculation will also be determined.

**AFOSR grant:**  
**and ASSERT grant:**

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**Final Technical Report, 1994-1998**

**Effect of Microbial Trophic Interactions**

**on the**

**Fate and Mobility of Soil Contaminants.**

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## Statement of Work

**Year 1.** This project will be initiated by the establishment of a culture collection isolated from contaminated drag strip soil (DSS) and clean Hudson River Sediment (HRS). Careful isolation, characterization, and long term maintenance of these bacteria and protists is critical for the success of the project. Bacteria will be characterized by sole carbon source utilization as well as standard morphological and chemical characteristics. Clonal cultures of protists will be identified by staining of morphological features for light microscopy, and characterized for their feeding and growth on the bacterial isolates obtained. Stable consortia of bacteria and protists in biphenyl cultures will be established and characterized. Retrieval of frozen consortia of bacteria and protists will be assessed. In addition, protists will be characterized for their sensitivity to biphenyl and Aroclors®, and assayed for acquired resistance. Studies of sorption and transfer of Aroclors® in bacteria and protist cells will be conducted. This very basic microbial ecology work is time consuming, but is essential to lay the ground work for future experiments. Analysis of the role of protists in *situ* biodegradation will begin with inhibition and/or stimulation of native bacteria and protist populations. Experiments to determine the fate of *Alcaligenes eutrophus* H850 in soil samples with and without protists will also begin. The effects of nutrient limited growth and predation pressure as pre-adaptations to inoculation will also be determined.

**Year 2.** Consortia of bacteria and protists obtained by biphenyl enrichment will be assessed for PCB degradation activity in sterile and non-sterile soil. Experiments will be initiated with simple constructed consortia of bacteria and protist from cultures, and tested for organic material processing and PCB degradation. Studies of degradation of PCBs by native bacteria and protist populations will focus towards maximizing activity by addition of supplemental substrates, organisms, and nutrients. Analysis of data and compilation of results into publications will be done.

**Year 3.** Work with artificially constructed microbial consortia will expand, building on information from previous work. Variables to be modified in these systems include combinations of different organisms, use of different alternate substrates, and different substrate concentrations, fluid phase vs. soil or vermiculite as surface area matrices, and nutrient additions. Analysis of data and compilation of results into publications will continue.

## Status of the Research Effort

### Introduction

This final report consists of manuscripts to be published in the scientific literature and abstracts of presentations made at national and international scientific conferences. In addition, the dissertation/thesis proposals and partially finished theses of graduate students supported on the grants are included. These will also result in publications as they are completed.

### Anticipated Publications. Theses and Dissertations (attached manuscripts)

Steffensen, W.S & R.A. Snyder. (projected). Effect of phosphate on MPN enumeration and culture of bacterivorous protists. Appl. Environ. Microbiol.

Snyder, R.A. & Black, M.D. (projected). Growth responses of the bacterivorous amoebae *Vanella* sp. on surfaces. J. Euk. Microbiol.

Snyder, R.A., Steffensen, W.S., Bauer, S., Wilkinson, S. and Tomas-Cody, T. (projected). Microbial nutrient in bioremediation. Appl. Environ. Microbiol.

Snyder, R.A. & W.S. Steffensen., & W. Gough. (projected). Bacteria and protists in contaminated sites. Chemosphere.

Frontera-Suau, R., F.D. Bost, T.J. McDonald, & Morris, P.J. (projected). Biodegradation of undegraded and partially degraded bonny light crude oil. Appl. Environ. Microbiol.

Bost, F.D., R. Frontera-Sau, T.J. McDonald, K.E. Peters, & P.J. Morris. (projected) Biodegradation of heavy venezuelan crude oils. Organic Geochemistry.

Rawlin, S. and P.J. Morris (projected) Effect of growth stage and nutrient concentration on bacterial cell surface properties. Appl. Environ. Microbiol.

Rawlin, S. (dissertation proposal) Untitled

Bost, F.D. (dissertation proposal). Soil and substrate effects on Microbial populations present in Jet fuel degrading enrichment cultures.

Frontera-Sau, R. (thesis proposal). The effect of crude oil composition on crude oil-degrading microbial communities.

Nicola, P. (thesis). Phospholipid retailoring in *Tetrahymena*: response to hydrophobic toxicants. (defends July 1998)

Tomas-Cody, T. (thesis nearly complete). *Tetrahymena pyriformis* response to and metabolism of *Burkholderia cepacia* lipopolysaccharide.

### Professional Personnel

Name	Dates	Affiliation
Richard A. Snyder, Ph.D.	Jun 94 - Apr 98	UWF
Pamela J. Morris, Ph.D.	Jun 94 Apr 98	MUSC
Samuel Rawlin Grad Student	Sept. 94 -	MUSC
Tai Huynh, student assistant	Jun 94 - May 96	UWF
Sherry Wilkinson, B.S. Technician	Jun 96 - Jun 96	UWF
John Millward, B.S. Technician	3 Jun. 96 -Feb. 98	UWF
Steven Bauer, Student Assistant	Apr 96 - Aug. 97	UWF
Megan Black, Student Assistant	Apr. 97-Aug. 97 -	UWF
F. Daniel Bost	Oct 97 - Apr 98	MUSC

### ASSRT Funding

Paula Nicola, Grad Student	Aug. 95 - Jun 98	UWF
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William Walker, Student Assistant	Apr. 97-Aug. 97 -	UWF
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Theresa Tomas-Cody, Graduate Student	Aug. 97 - Jun 98	UWF
Wade Gough, Graduate Student	Aug. 97-Oct. 97	UWF